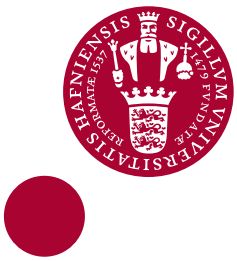

Astrocytes take the stage in a tale of signaling-metabolism coupling

Bak, Lasse K

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Astrocytes take the stage in a tale of signaling-metabolism coupling

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Lasse K. Bak[1]

From the Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, 2 Universitetsparken, 2100 Copenhagen, Denmark

Edited by Jeffrey E. Pessin

Astrocytes are crucial cells in the brain that are intimately coupled with neuronal metabolism. A new paper from San Martín *et al.* provides evidence that physiological levels of the gaseous signal molecule NO can rapidly and reversibly increase astrocyte metabolism of glucose and production of lactate. A proposed neurological coupling—from the potential source of NO, endothelial cells, to the potential beneficiary from the lactate, neurons—prompts new questions regarding the controversial role of lactate in the brain.

Astrocytes make up a large part of the human brain, and because of their many processes, extended feet that attach to axons, dendrites, and capillaries in the brain, they sometimes appear to be star-shaped. However, their reputation as the stars of the brain also derives from the fact that they play a key role in numerous physiological processes and are intimately coupled with neuronal function and metabolism (1, 2); so intimately, in fact, that we in our lab sometimes refer to neurons as AHCs, astrocyte helper cells. Ever since Pellerin and Magistretti made the suggestion in 1994 (3) that astrocytes provide lactate as metabolic fuel for neurons, there has been much interest in how astrocyte energy metabolism is regulated. Answering this question has both fundamental and practical importance; for example, interpretation of positron emission tomography imaging signals from fluorodeoxyglucose depends on our understanding of the underlying cellular activity dictating the measured responses. While the overall topic of astrocyte-neuron coupling via the lactate pool is controversial, being debated at every conference in the field and in the literature (*e.g.* Refs. 4, 5, and 6), other basic questions regarding astrocyte metabolism remain unresolved. In this context, San Martín *et al.* (7) provide a new clue into possible mechanisms of cellular cross-talk in the brain in their investigation of NO's influence on astrocytes.

Blood flow determines the amount of glucose delivered to the brain, and NO originating from endothelial cells is known to be one of several potent signaling molecules involved in the regulation of vascular tone or the extent to which blood vessels are dilated. Glycolysis is the conversion of glucose to pyruvate, and previous work has shown that NO may increase the flow through this pathway, *i.e.* glycolytic flux, by blocking complex IV, which inhibits mitochondrial respiration and thus induces AMP-activated protein kinase (AMPK)-dependent phosphorylation of the phosphofructokinase-2/fructose-2,6-bisphosphatase (PFKFB)[2] dual enzyme (8). What was not clear at that time was whether the effects of NO would be present at nanomolar rather than micromolar levels (*i.e.* closer to what may be physiological) of NO, and whether these effects were readily reversible.

The authors test these questions using a range of approaches. First, employing mixed cultures of neurons and astrocytes, the authors confirm that a 1-min exposure to 2 μM NO induces an immediate decrease in the glucose level and an increase in the level of lactate; both were fully reversible within a couple of minutes after removal of NO from the buffer. In this assay, a decrease in the cytosolic level of glucose is interpreted as an increase in glycolytic flux. Next, the authors investigated whether the effect of NO on cytosolic glucose levels would be more pronounced at a lower level of O_2, since that would indicate that mitochondrial respiration via complex IV was involved in the mechanism, and not other targets of NO that are not directly dependent on respiration. This was indeed the case, and at the lower level of O_2, the recovery back to baseline upon withdrawal of NO was slower. In some cells, prolonged exposure to 2 μM NO (3 min) even caused a persistent decrease in cytosolic glucose levels consistent with continuing inhibition of complex IV, possibly by nitrosylation. However, whether the activity of PFKFB is involved in the mechanism, as suggested previously (8), was not explored in this investigation.

One drawback of employing the glucose biosensor is that it reports free, non-phosphorylated glucose levels, not glycolytic flux *per se*; thus, other changes to glucose concentration, such as from glucose transporter activity or changes in glycogen metabolism, may influence the read-out. The authors performed experiments in the presence of a blocker of glucose transport to rule out this complication. Under this experimental condition, the authors found significant effects of as low as 100 nM NO in some cells suggesting that nanomolar levels of NO may indeed modulate glycolytic flux (or glycogen metabolism); future work to deconvolute the influence of glycogen metabolism will be a welcome addition to this line of research. In a second experiment, the authors employed a biosensor that measures cytosolic levels of pyruvate, the direct precursor of lactate, and switched the buffer to contain only pyruvate as

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[1]To whom correspondence should be addressed. Email: laba@sund.ku.dk.

[2] The abbreviation used is: PFKFB, phosphofructokinase-2/fructose-2,6-bisphosphatase.

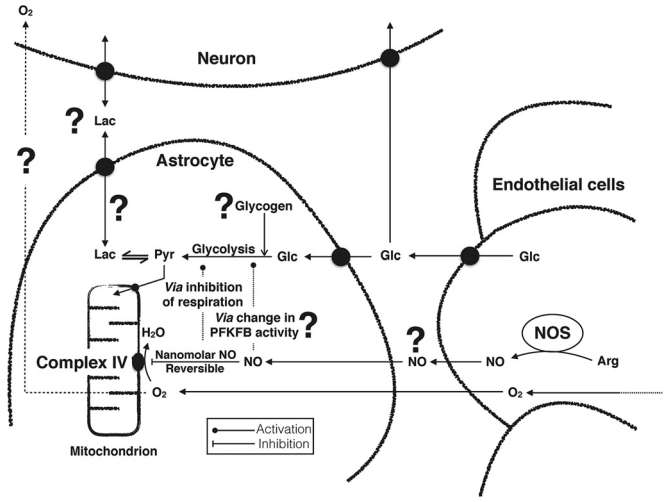

Figure 1. Glycolytic activity in astrocytes. Glycolytic activity in astrocytes may be regulated by release of NO from nearby endothelial cells, as suggested by San Martín *et al.* (7) and explained in the text. The novel contribution by San Martín *et al.* (7) is that the increase in glycolytic flux mediated via inhibition of complex IV by NO is reversible and can be induced by nanomolar levels of NO, as indicated. However, several open questions remain as indicated by *question marks*. It should be noted that the actual physiological levels of NO and how far it reaches from the point of origin is still not known in detail. *NOS*, nitric-oxide synthase.

substrate. After establishing a steady-state level of cytosolic pyruvate (*i.e.* uptake and mitochondrial consumption is presumed to match), a blocker of cellular uptake of pyruvate was introduced, and the disappearance of pyruvate was monitored and assumed to correspond to mitochondrial consumption. In some cells, the decrease in pyruvate was curbed by the presence of as low as 50 nM NO, suggesting that NO at these low levels inhibits consumption of pyruvate consistent with inhibition of respiration.

The work by San Martín *et al.* (7) provides a proof-of-principle that NO at nanomolar levels, presumed to be within the physiological range, can reversibly increase cellular glycolytic flux by inhibiting mitochondrial respiration. As mentioned above, further work is needed on this topic to determine whether the mechanism involves PFKFB or glycogen metabolism.

In addition to these data, the authors put forward a proposal for the functional significance of their observations. Because astrocytes themselves do not express any of the known isoforms of nitric-oxide synthases, whereas both endothelial cells and neurons do, they appear to be targets rather than producers of NO. This prompts the authors to suggest that NO released from endothelial cells during increased blood flow in turn spares O_2 for use in neurons and increases glycolytic flux and lactate production in astrocytes and that this aids in maintaining an extracellular lactate pool available to neurons (Fig. 1). As hinted above, the existence of this pool is in itself controversial because, among other factors, there is disagreement as to whether there is a unidirectional flow of lactate in the intact brain (a necessary component of the lactate shuttle hypothesis). At the moment, therefore, any regulatory role of endothelial NO on astrocyte energy metabolism remains an open question. There is an intriguing putative link between endothelial NO production through dilation of blood vessels and thus an elevated supply of glucose to the brain to an increase in glycolytic flux and production of lactate in astrocytes. While San Martín *et al.* (7) has provided proof-of-principle that NO regulates astrocyte glycolysis, experimentation employing the emerging imaging systems surgically attached to behaving animals will ultimately be needed to fully resolve this question. Finally, we and others (9) have also observed a form of activity-dependent reversible increase in glycolysis and lactate production in neurons albeit via a different mechanism. Thus, an alternative to the lactate shuttle hypothesis could be that both neurons and astrocytes produce lactate during brain activation and that this lactate pool can then be metabolized at a later point by both cell types (and maybe other cells) or dispersed within the brain parenchyma. However the story of neuronal coupling ends, these new data from San Martín *et al.* (7) provide an interesting chapter that advances our understanding of astrocyte metabolism.

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